Survival and growth of juvenile freshwater mussels (Unionidae) in a recirculating aquaculture system

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Abstract: An indoor recirculating aquaculture system was constructed to provide suitable conditions to culture juvenile freshwater mussels. In the first of three growth trials, *Villosa iris* (I. Lea, 1829) juveniles were cultured for 22 wk, and grew from an initial mean length of 0.4 mm to 2.7 mm. Survival was 26.8% overall. In the second trial, growth and survival were compared between juveniles of *V. iris* held in sediment and without sediment. The initial mean length of both groups was 2.7 mm, and this experiment ran for 17 wk. The juvenile mussels in sediment grew to a mean length of 5.7 mm with 85% survival, significantly greater ($p < 0.01$) than juveniles held without sediment (4.5 mm, 74% survival). In the third trial, two cohorts of juvenile *Lampsilis fasciola* Rafinesque, 1815, increased in length from 1.1 mm and 1.4 mm to 3.3 mm and 4.1 mm, respectively, with comparable survival (78.7% versus 64.5%). Results of these trials demonstrate that juvenile mussels can be reared successfully within recirculating systems. One of the factors deemed important in successful culture is continuous feeding of an appropriate food source. In this study, a unialgal culture of *Neochloris oleabundans* Chantanachat and Bold, 1962, was used throughout. Regular cleaning of the system and water replacement also was important. Finally, the culture of juveniles in sediment appears to be an important factor in ensuring good growth and survival. This phenomenon could be related to pedal feeding behavior, proper orientation of the mussels for filtering efficiency, or stability from physical disturbance.

Key words: Unionidae, freshwater mussels, *Villosa*, *Lampsilis*, algae, recirculating aquaculture

Major declines in freshwater mussel populations (Unionidae) were recorded by the early twentieth century (Smith, 1899; Coker *et al.*, 1921) and were attributed primarily to overfishing for a burgeoning pearl button industry (Coker, 1919). More recently, declines in unionid numbers have been noted and ascribed to pollution, habitat destruction or alteration, competition from non-indigenous bivalve species [*e. g.* Asian clam, *Corbicula fluminea* (Müller, 1774), and the zebra mussel, *Dreissena polymorpha* (Pallas, 1771)], as well as to harvest for shell to produce nuclei for pearl culture. The realization of this dramatic decline by resource managers and scientists within the last two decades has prompted an increase in much needed studies on basic life history and ecology of numerous unionid species. Such studies continue as more is learned of environmental and host-fish requirements for survival and reproduction in this group of bivalves (Isom and Hudson, 1982; Zale and Neves, 1982; Neves and Widlak, 1987; Dimock and Wright, 1993).

Historically, reports on the aquaculture of juvenile unionids have conveyed little detailed empirical information. Many of the data are anecdotal, with little or no monitoring of the progress of the juveniles throughout the culture period (Lefevre and Curtis, 1912; Coker *et al.*, 1921; Howard, 1922). Culture attempts on unionids using artificial media have resulted in some success (Isom and Hudson, 1982; Keller and Zam, 1990). However, the application of these results to the large-scale and long-term propagation of unionids remains speculative and untested. Artificial culture of endangered and threatened mussel species has been strongly recommended in recovery plans as a strategy to enhance declining population numbers, as well as for the reintroduction of species to sites within their historic ranges.

With these propagation needs in mind, we initiated a project focusing on factors influencing the growth and survival of juvenile unionids in a captive environment. To this end, a recirculating system was constructed to rear juvenile mussels under controlled conditions of food rations, water temperature and flow, and to monitor growth

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and survival. This report describes feeding and maintenance protocols for the recirculating culture system and presents results of growth and survival trials with juvenile mussels.

MATERIALS AND METHODS

The recirculating culture system was located within a greenhouse facility at the Virginia Tech Aquaculture Center, Blacksburg, Virginia. The greenhouse has the capability of maintaining fairly constant temperature either by the use of an evaporative cooling system or propane heaters. However, water temperatures throughout the study, while differing from ambient temperatures especially in winter months, exhibited some seasonal fluctuation.

The recirculating system used for culturing juvenile mussels consisted of a rectangular reservoir tank (225 l) from which water was pumped by a 1/25 hp magnetic drive pump through half-inch PVC line, to one end of an elongated raceway-type tank, which served as the primary culturing chamber. The raceways were 3 m in length and 66 cm in width. Water flow into the raceway was regulated and measured using an in-line flow meter. Water was introduced into the raceway by ten parallel jets across the width of the chamber, which provided adequate uniformity in flow within the system. The water in the raceway was gravity-fed back to the reservoir via a standpipe. Water capacity in the raceway could be varied by adjusting the height of the standpipe. Aeration in the system was provided by air pumped into the reservoir and supplemented by the pumping action. Throughout the growth trials, the water depth in the raceway was 20 cm, giving a volume of 170 l.

Municipal water was conditioned (dechlorinated) by aeration for a period greater than 24 hr prior to use in the culturing system. Because the municipal water was soft (< 55 mg/l $CaCO_3$), hardness of the water was increased by the addition of well-water (hardness of 450 mg/l $CaCO_3$) to maintain an overall hardness in the system at ca. 200 mg/l $CaCO_3$.

Throughout the growth trials, juvenile mussels were fed the unicellular green alga *Neochloris oleoabundans* Chantanachat and Bold, 1962, which has been shown to support good growth in juvenile mussels (Gatenby, 1994). Mussels were fed algae at a density of 10,000 cells/ml either once or twice daily. Ultraviolet-sterilized and dechlorinated water was used for algal cultures and the recirculating system. Living algal cultures were maintained semi-continuously in 250 l Kalwall clear plastic tubes (Kalwalls Aquacenter, Leland, Mississippi). The unialgal cultures were not axenic. Enrichment of the algal culture was achieved by the addition of appropriate quantities of nutrient media (Ukeles, 1971). We attempted to harvest algae at the late exponential growth phase, although this might not have been achieved for all feeding events.

Mussel Species

The growth trials described herein were conducted on common species of mussels to determine the potential success of such a system on endangered species. One surrogate for these rare species is the rainbow mussel, *Villosa iris* (I. Lea, 1829), common to streams and rivers of the upper Tennessee River system. This species was selected because of its relative abundance, and the success with which large quantities of juveniles can be acquired from host fishes. Also, the habitat requirements of *V. iris* (riffles and shoals) are similar to those of many endangered species. In addition, two cohorts of the wavy-rayed lampmussel, *Lampsilis fasciola* Rafinesque, 1820, were utilized in growth trials. This species also is common to the upper Tennessee River system. Fish infestation and collection of juveniles were accomplished by standard techniques (Zale and Neves, 1982). Rock bass (*Ambloplites rupestris* Rafinesque, 1817) were infested with glochidia of *V. iris*, and largemouth bass (*Micropterus salmoides* Lacépède, 1802), were used as the host fish for *L. fasciola*.

Growth Trial 1

Young rainbow mussels with a mean shell length of 0.4 mm were placed in three petri dishes (18 cm x 1 cm) with 600-700 mussels per dish. Fine sediment (< 120 µm), collected from the Clinch River at Carbo, Virginia, and aerated during storage, was placed in each dish to a depth of 1-1.5 mm. The introduction of feed to the mussels in this first trial was not regimented, as the primary purpose was to fine-tune the system, in terms of adjusting flow and ensuring good circulation. The flow rate of water through the raceway was 6 l/min. Growth (shell length) was assessed at 2, 4, 8, 16, and 22 wk of the trial from a sample of juveniles seived from the sediment. Fresh sediment was placed in the culture dishes after each sampling event. Water was changed in the system every 10-12 d (with one exception). This pilot study was initiated in August and terminated in December 1996.

Growth Trial 2

A second trial followed the first, using those mussels surviving the first trial supplemented with mussels of the same age from another study. Given the relatively small number of animals held in the system, it was anticipated that water quality within the system would not be seriously compromised. However, water was changed on a weekly basis and water quality variables were more closely monitored in this study. On a weekly basis, temperature, pH, hardness, un-ionized ammonia, and dissolved oxygen were measured. Also, older juveniles require a greater rate of

water change (flow) than younger individuals (Jiang Li-Fan, Freshwater Fisheries Research Center, Wuxi, P. R. China, pers. comm.), therefore, flow rate across the dishes was increased to 9 l/min.

Two culture techniques were evaluated within the system during this trial. The first culture method employed a sediment substratum (< 350 µm). Three replicate petri dishes contained sediment to a depth of 0.5-0.7 cm. The growth of juveniles in these dishes was compared to that of juveniles in dishes (N = 3) without substratum. The rationale for this comparison was to determine whether sediment was a necessary component in the survival and growth of juvenile mussels at this particular size (age) in their development. If not, then sediments could be discarded from the culture protocols, thus making maintenance and cleaning of the culture system much easier. Upon initiation of the second growth trial, 100 juveniles were placed within each petri dish. Growth (length of 30 juveniles per dish) and survival (total living count per dish) were monitored every 2 wk for the duration of the study (17 wk).

Growth Trial 3

Two cohorts of juvenile *Lampsilis fasciola* were placed in the recirculating system to evaluate their growth and survival with the rearing protocols previously described. At the start of this trial, cohort 1 had a mean size of 1.1 mm. Growth and survival were estimated four times over the subsequent 12 wk. Cohort 2 had a mean size of 1.4 mm upon initiation of this trial, and these mussels were sampled only twice during the 12 wk period.

RESULTS

Growth Trial 1

Juvenile mussels in the three dishes, at an initial mean shell length of 0.4 mm, exhibited a steady rate of growth for the 22 wk period, achieving a mean length of 2.7 mm (Fig. 1). Survival after the first two sampling periods (4 wk) was high (approx. 95%) in all dishes. However, between the second and third sampling interval (8 wk), high mortality (60-65%) was observed in all dishes. Thereafter, mortality was low, concluding with an overall survival of 26.8%. Water temperatures in the dishes from October to December ranged from 24.5-15.4°C, with a steady decline as winter progressed.

Growth Trial 2

The mean lengths of juvenile mussels at the start of growth trial were identical (mean = 2.7) for the sediment and no-sediment treatments (Fig. 2). Growth for the first two sampling periods (4 wk) was slow for both treatments;

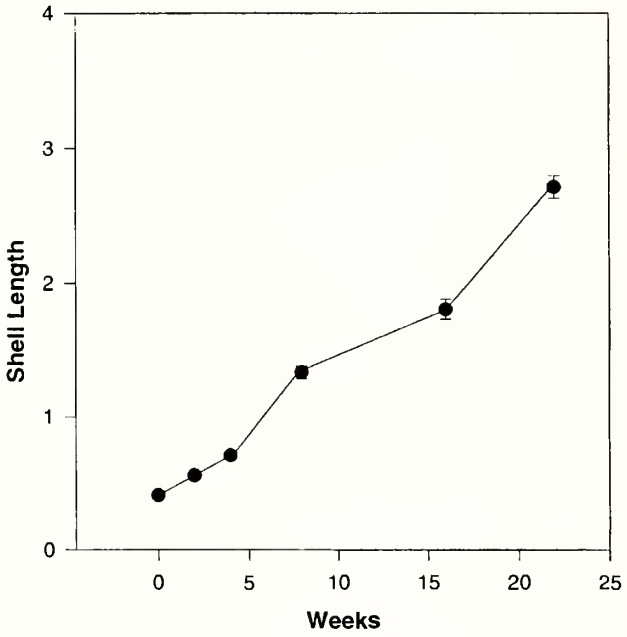

Fig. 1. Mean shell length (mm ± 2 SE) of juvenile rainbow mussels, *Villosa iris*, over 22 wk (Trial 1).

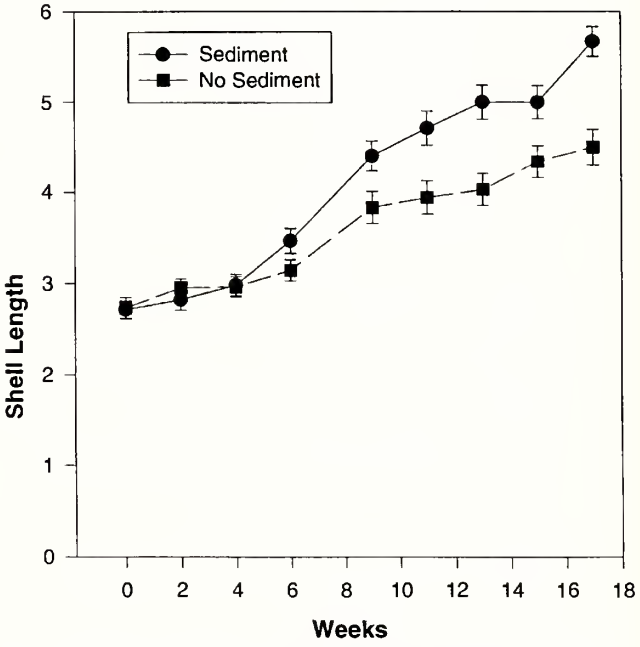

Fig. 2. Mean shell length (mm ± 2 SE) of juvenile rainbow mussels, *Villosa iris*, in sediment and no-sediment treatments over 17 wk (Trial 2).

however, after 6 wk, a discernible difference was apparent between the two treatments. The mean size of juveniles in the sediment treatment was 3.5 mm, whereas that of juveniles in the no-sediment treatment was 3.1 mm. This difference was further exaggerated after 9 wk, when the mussels in the sediment and no-sediment treatments had mean sizes

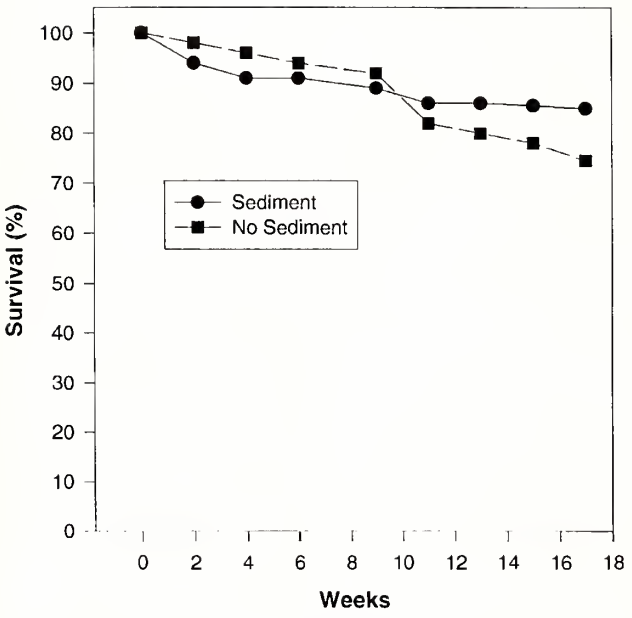

Fig. 3. Mean survival of juvenile rainbow mussels, *Villosa iris*, in sediment and no-sediment treatments over 17 wk (Trial 2).

of 4.4 mm and 3.8 mm, respectively. Upon completion of the study at 17 wk, the mussels held in sediment were 5.7 mm; no-sediment mussels had attained a mean size of only 4.5 mm. Repeated measures Analysis of Variance on these data, after termination of the study, indicated that the mussels held in sediment were significantly larger than those held without sediment ($p < 0.01$). Overall, survival also differed between the two treatments (Fig. 3). Those juveniles held in sediment had an overall survival of 85%, whereas the no-sediment treatment mussels exhibited significantly lower survival (74%). It was noted throughout this study that many juveniles held in sediment attached to the container surface and each other by byssal threads. This trait made separation for measuring and enumeration difficult. Conversely, those mussels cultured in dishes without sediment produced no byssal threads at any period in the study.

Growth Trial 3

Cohort 1 had grown to a mean size of 2.3 mm after 4 wk and 3.3 mm after 12 wk, when the trial was terminated (Fig. 4). Cohort 2 had grown to 3.9 mm after 10 wk and to 4.1 mm by 12 wk. Survival of cohort 1 and cohort 2 after 12 wk was 78.7% and 64.5%, respectively.

Water Quality

Water temperatures throughout the study fluctuated with air temperatures and ranged between 15.4°C and 26.4°C. The overall mean water temperature in the culture system was 20.6°C. Dissolved oxygen (mg/l) and pH values remained relatively stable throughout the study; both had mean values of 8.3. Un-ionized ammonia showed some variability, ranging from 0 to 0.053 mg/l, with a mean value of 0.013 mg/l. Water hardness had a mean value of 213 mg/l $CaCO_3$, close to the targeted value of 200 mg/l $CaCO_3$. However, there was considerable variation in hardness values between samplings (range 138-277 mg/l $CaCO_3$).

DISCUSSION

The growth of *Villosa iris* in Trial 1 was notable; 2.7 mm after 22 wk. This rate of growth exceeds that of 2.9 mm after 39 wk (Gatenby *et al.*, 1996), and 1.8 mm after 20 wk under different culture conditions for the same species (Gatenby *et al.*, 1997). The rainbow mussel is a small, slow-growing species in the Tennessee River system. When compared to other estimates of growth rates observed in the wild, the growth rate and sizes recorded in this study were considerably greater than those previously reported. Neves and Widlak (1987) estimated the mean size of juvenile freshwater mussels from a variety of species (including *V. iris*) as 2.7 mm after 1 yr (52 wk). Survival of our juvenile mussels also was comparable to those reported in previous studies. Gatenby *et al.* (1996) reported 8% survival of *V. iris* after 165 days, and 30-38% survival after 140 days under their best culture conditions (Gatenby *et al.*, 1997). After 154 days, the mean survival in growth trial 1

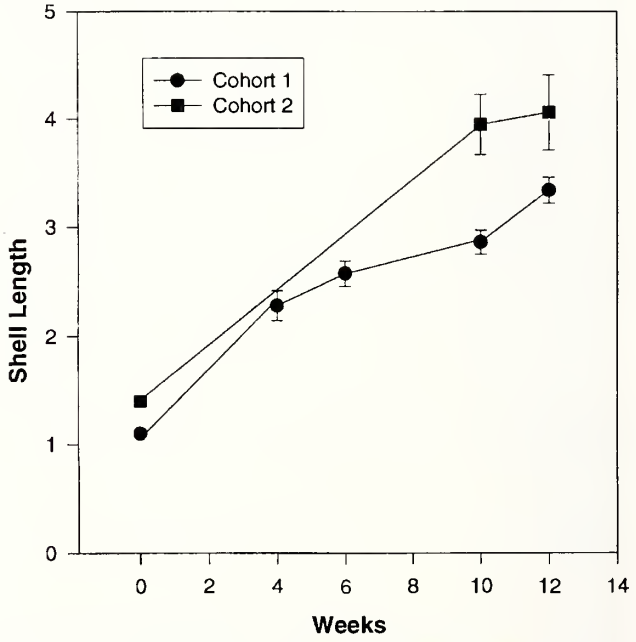

Fig. 4. Mean shell length (mm ± 2 SE) of two cohorts of the wavy-rayed lampmussel, *Lampsilis fasciola*, over 12 wk (Trial 3).

was 26.8%, which included the large single mortality event that occurred between the second and third sampling periods. The exact cause of this high mortality event is unknown; however, it is our opinion that the cause was poor water quality due to an oversight. Because the scheduled water change did not occur between the second and third sampling periods, the sediments were fouled with filamentous algae, previously shown to result in high juvenile mussel mortality (Yang, 1996). The water quality condition in the interstitial sediments, while unknown, was also assumed to be less than optimum. Juveniles of even eurytopic species such as *Utterbackia imbecillis* (Say, 1829) and *Pyganodon cataracta* (Say, 1817) have been shown to be very sensitive to conditions of hypoxia and low pH, as well as elevated temperatures (Dimock and Wright, 1993).

The performance of juvenile mussels in growth Trial 2 also proved informative. The significantly greater growth of mussels held in sediment as compared with those with no sediment was somewhat surprising. We compared growth and survival of juvenile rainbow mussels cultured with and without sediment in this trial. If comparable or better growth was achieved without the sediment, then the use of sediment could be dispensed with, which would make the maintenance and cleaning of the culture systems easier. However, this was not the case. Those animals held in sediment had greater growth and survival than those juveniles with no substratum at all. The reason for this phenomenon is unclear; however, we offer some possible reasons for the differences observed. Juvenile mussels could obtain nutrition from the sediment, to include organic matter or micro-organisms. The substratum also allows juvenile mussels to orient themselves properly for feeding. By doing so, they can siphon from higher in the water column and avoid the slower water in the boundary layer near the sediment-water interface (Vogel, 1981; Mann and Lazier, 1991). The mussels in the sediment were clearly seen to orient themselves in this manner, whereas the mussels without sediment lay on one valve with no vertical orientation.

The sediment offers a certain amount of stability, which would buffer juvenile mussels from disturbance by turbulent water flow or vibrations originating outside the culture vessels. Those juveniles held without sediment were subjected to greater disturbance, as witnessed by the buffeting (from both flow and vibrations) that they received in the dishes. When disturbed in such a manner, the mussels had a tendency to cease feeding and close up. This cessation of feeding could result in their slower growth and reduced survival. The response of the wavy-rayed lampmussel cohorts in growth trial 3 seems to support this disturbance hypothesis (Fig. 4). Both cohorts appeared to have similar growth rates until the first sampling of cohort 1. Thereafter, the growth of this cohort appeared to decrease dramatically with further samplings. The first sampling of cohort 2 did not occur until week 10, after which the growth rate decreased appreciably. Sampling involved removal of the mussels from water and much handling for periods in excess of 20 min. Stress associated with the handling of juvenile mussels could have contributed to a reduction in growth. We speculate that disturbance associated with the sampling of these juveniles (measuring and counting) had a detrimental effect on the mussels. The juvenile mussels held without sediment would undoubtedly be exposed to greater disturbance, and this could be reflected in reduced growth rate, as we observed. Vanderploeg *et al.* (1995) determined that filtration rate in *Lampsilis radiata siliquoidea* (Barnes, 1823) was comparable between mussels in sediment and those lying on their sides. However, they used adult mussels and warned that such a phenomenon might not apply to all unionids. It has long been recognized that filtration rate in bivalves is adversely affected by mechanical or physical disturbance (Jorgensen,1960; Mohlenberg and Riisgard, 1979).

The degree of stress that young mussels experienced in our study can also be evidenced by the fact that the mussels held in sediment produced copious amounts of byssal threads to secure themselves to the bottom of the culture dish. Conversely, the mussels held without sediment produced no byssal threads. After the study was completed, the mussels held without sediment were placed in sediment, and within 1 wk they produced byssal threads. We hypothesize that the sediment provided appropriate habitat for the mussels, which consequently produced byssal threads to secure themselves within the substratum. Those held without sediment were in unstable habitat and did not produce byssal threads. A conclusion of our study is that young freshwater mussels, without suitable substratum, do not exhibit high growth and survival. This is supported by evidence from marine mussels, *Mytilus* spp., where production of byssal threads is mediated by the favorability of the habitat encountered (Morton, 1992), and is a result of net energy surplus (Hawkins and Bayne, 1992).

The water quality parameters recorded throughout the study were not detrimental to juvenile mussels (Table 1). Temperature fluctuations, despite their range, were gradual and well within the range that juvenile mussels experience in the rivers and streams of southwestern Virginia. Hardness levels also fluctuated considerably, yet the values were such that the water was considered moderately hard (Landau, 1992). Dissolved oxygen and pH values remained relatively stable and were not deemed stressful to the juveniles mussels. Un-ionized ammonia levels were considerably lower than levels reported to cause mortality in the marine northern quahog, *Mercenaria mercenaria* (Linné, 1758) (see Stevens, 1982).

Despite the differences observed in growth Trial 2, the growth and survival in both treatments with *Villosa iris*

Table 1. Summary of water chemistry during juvenile mussel culture experiments.

Date	Temperature (°C)	Dissolved Oxygen (mg/l)	pH	Un-ionized Ammonia (mg/l)	Hardness (mg/l $CaCO_3$)
10/25/96	21.0	7.8	8.5	0.013	255
10/31/96	24.6	7.4	8.4	0.008	138
11/5/96	21.7	7.9	8.4	–	160
11/8/96	22.5	7.9	8.2	–	–
11/14/96	15.4	9.1	8.2	–	250
11/18/96	20.0	7.6	8.6	–	–
11/22/96	20.8	8.6	8.7	0.037	235
11/30/96	17.4	9.3	8.6	0.022	235
12/14/96	19.7	9.3	8.5	0.007	205
12/22/96	15.7	9.6	8.8	0.000	193
12/28/96	23.1	8.6	8.6	0.043	243
1/4/97	26.4	7.5	8.9	0.053	249
1/11/97	18.9	9.4	8.6	0.040	206
1/18/97	15.9	10.9	8.4	0.000	220
1/24/97	17.9	8.9	8.1	0.002	201
2/2/97	23.0	8.4	8.4	0.001	190
2/8/97	19.5	8.7	8.0	0.001	201
2/16/97	17.2	9.9	8.5	0.008	277
2/21/97	24.6	7.5	8.4	0.003	264
3/7/97	19.5	–	–	–	200
3/16/97	17.9	8.8	8.3	0.025	190
3/21/97	24.0	5.8	8.3	0.001	167
3/31/97	18.3	9.0	7.3	0.000	144
4/4/97	22.5	6.1	8.0	0.001	230
4/14/97	20.9	6.5	7.8	0.001	261
4/22/97	20.9	7.9	7.5	0.000	215
Mean ± SD	20.4 ± 2.9	8.3 ± 1.2	8.3 ± 0.4	0.013 ± 0.0016	213 ± 37.9

were excellent, and matched or exceeded the rates observed in previous culture attempts (Yang, 1996; Gatenby *et al.*, 1997). The commercial culture of marine bivalves is considered successful if a survival rate of 1-5% is realized from egg to planting size (M. Castagna, Virginia Institute of Marine Science, Eastern Shore Laboratory, pers. comm.; M. Pierson, Cherrystone Aquafarms, Cheriton, Virginia, pers. comm.). Our culture attempts compare favorably with these estimates. However, it must be noted that brooding organisms (*e. g.* freshwater mussels) tend to exhibit higher survival than non-brooding planktotrophic species (*e. g.* many marine bivalves). The growth and survival observed with the two cohorts of *Lampsilis fasciola* was encouraging, and provided evidence that the system and techniques employed in these studies can be applied successfully to other species of freshwater mussels. No single factor can be identified as causative in the success of our culture attempts. It is our opinion that the combination of the system (providing unidirectional consistent water flow with adequate dissolved oxygen levels) and the husbandry techniques (daily feeding and regular cleanings) were extremely important for the successful culture of the juvenile unionids. It has long been appreciated that successful culture of marine bivalves can only be achieved by vigilant monitoring, cleaning, and constant feeding through the larval and juvenile phases (Castagna and Kraeuter, 1981). The ability to consistently rear large numbers of freshwater mussels, from the juvenile stage to a size suitable for relocation to natural environments, is a much-needed step in the conservation of rare unionids or the production of commercially valuable species. Results of our study demonstrate that this goal is achievable with further research into the factors influencing the growth and survival of freshwater mussels within indoor culture systems.

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